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Gonadal sex differentiation in the fish, Oreochromis mossambicus

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ABSTRACT

The present work is an attempt to study the different stages involved in the development of gonads in the cichlid fish Oreochromis mossambicus. Histological studies of the gonad revealed the appearance of gonadal primordium in between the gut and pronephric duct in the developing stages from 2 day hatchling to30 day hatchling. As per the observations made up to 30 days there is an increase in the gonadal analogue. The gonial cells at 20 day show some meiotic structure which initiates the sex differentiation, and shows sex differentiation in this species may takes place after 20 days of fertilization.

Keywords: Oreochromis mossambicus, gonadal analogue, meiosis, sex differentiation.

1. INTRODUCTION

Among all the vertebrates, fishes exhibit the most divergent display of sex (Atz, 1964). The functional sex reversal is a natural phenomenon in the teleosts. Various types of sexuality, from synchronous hermaphroditism to gonochorism have been reported in teleosts (Yamamoto, 1969 and Jelly man, 1976).

The origin of germ cells, mode of sex differentiation, development of gonads have been studied in some species of teleosts like Carassius auratus (Stromsten, 1931), Tilapia aurea (Eckstein and Spira, 1965) Channa punctatus (Belsare, 1966), Clarias batrachus (Belsare, 1974) Tilapia zilli (Yoshikawa & Oguri, 1978) Cyprinus carpio (Davies and Takashima, 1980) Salmo gairdneri (Hurk van den & slof 1981, Lebrun et al, 1982) Tilapia (Saratherdon), (Jalbert and zohar 1982), review work on various steps involved in gonad differentiation and development in some of the teleosts, (Nayak and Nadkarni, 1993)

Extensive studies are carried out in other tilapia like Oreochromis niloticus and O. aureus but the gonadal studies are relatively less in Oreochromis mossambicus. Except the work by Nakamura and Takahashi, (1973) and Nakamura, (2013) little is known about the development of gonads in the cichlid fish, Oreochromis mossambicus. Hence an attempt has been made to study the gonadal development till the sex differentiation in Oreochromis mossambicus.

Natural distribution

Tilapia belong to a large family of fish (Cichlidae) that are found naturally in the warm, fresh and brackish waters of Africa, South and Central America, Southern India and Sri Lanka. Oreochromis mosambicus is a continuous breeder, thrives well even in poor water quality and shows an elaborated parental care. It is a mouth brooder; eggs are externally fertilized, female parent picks up the eggs in

her mouth immediately after fertilization and keeps the eggs in her mouth for incubation until hatching and for several days even after hatching.

2. MATERIALS AND METHODS

In order to study the development of gonads in different developmental stages, the adult fishes were procured from the local tanks of Hubli Dharwad area and are kept in aquaria under laboratory conditions to acclimatize for about a week to 15 days. Breeding of Tilapia was conducted in two different aquaria in the ratio of (3:1) three females and one male in each aquarium under laboratory conditions providing all the necessary conditions and are fed with commercial food granules.

Female lays eggs and males release the milt thus fertilizing the eggs. Fertilized eggs are taken by the female in her mouth and it nourishes the eggs and hatchlings until yolk dissolves. It requires about one to one and half month for the breeding and to get the different developmental stages. Developing stages from the day one to 30 days with a gap of 5 days were collected and fixed in Bouins fluid for histological studies. Then they were dehydrated in ethyl alcohol grades and were subsequently embedded in the paraffin block. Serial transverse sections of 5 μm thickness were cut and stained with hematoxylin and eosin according to the standard procedures. Stained sections were observed for the gonadal development.

3. OBSERVATIONS

2 Day hatchlings

Serial sections observed showed the formation of small gonadal primordia in the posterior region of the abdominal cavity immediately below the kidney. These primordia are suspended from the dorsal peritoneal wall by short mesenteries in between the gut and pronephric duct. The large rounds to oval primordial germ cells (PGCs) were found in the gonadal primordia on both sides. These are distinct and are surrounded by somatic cells. The PGCs contain large and central nucleus with prominent nucleoli. They are much larger than the somatic cells. (Fig 1 and 2)

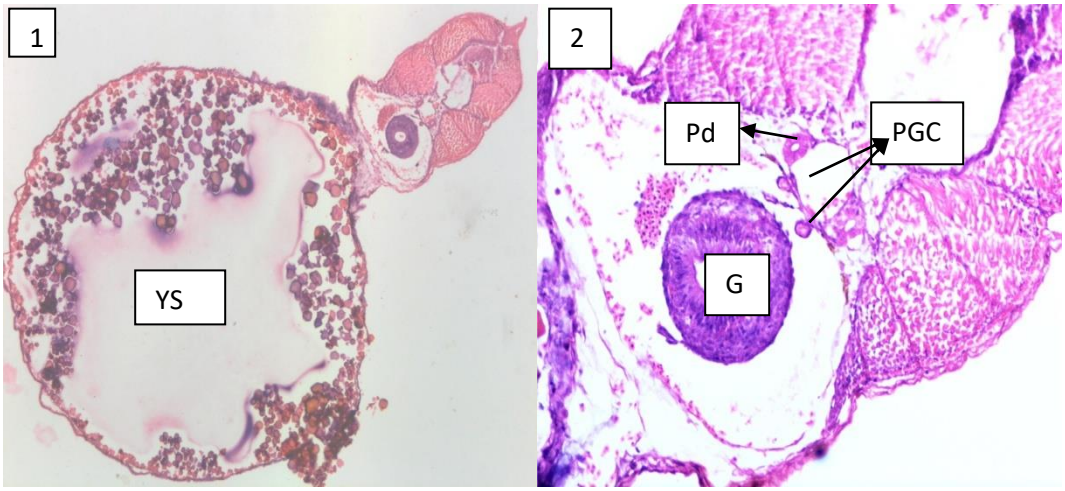

Figure 1 Whole section of two day hatchling, YS- Yolk Sac

Figure 2 Section of two day hatchling showing gonadal primordium, Arrow indicate Primordial germ cells PGC-Primordial germ cell, G, - Gut; Pd, - Pronephric duct.

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5 Day hatchlings

There is a slight increase in the size of the gonad and not much change in the histological structure of the gonad is observed. (Fig 3)

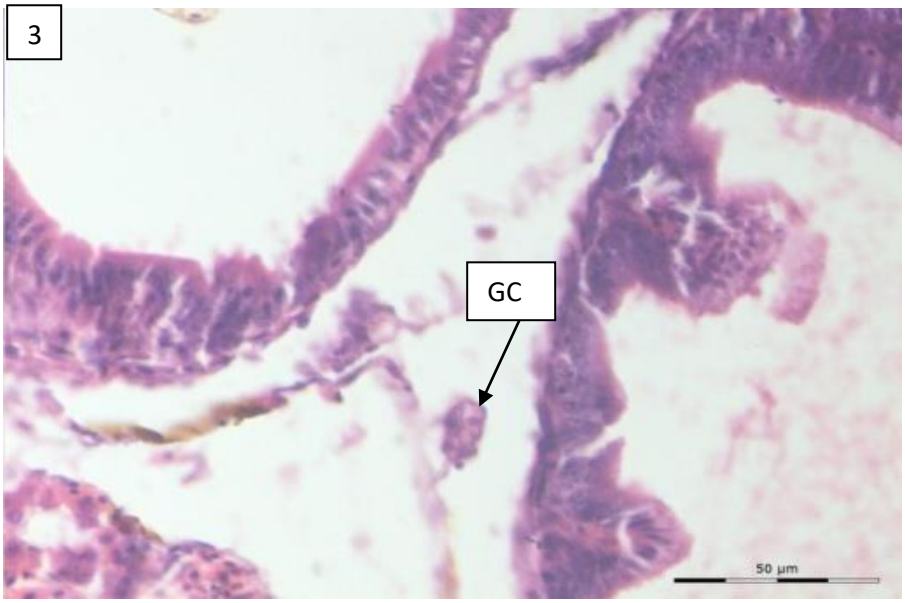

Figure 3 Section of Five day hatchling-gonad with germ cell (GC). Scale bar is 50 µm

10 Day hatchlings

Mitotic germ cells were observed and were surrounded by somatic cells of varying shapes. (Fig 4)

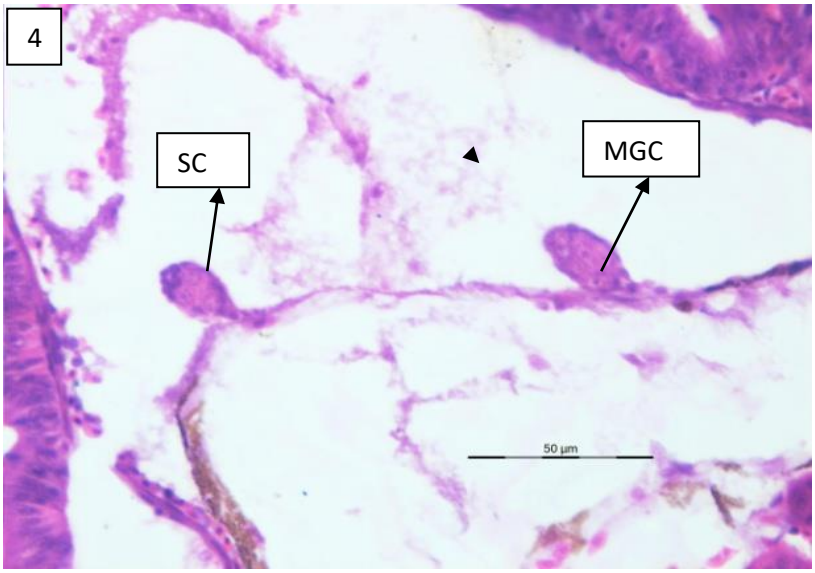

Figure 4 Section of 10day hatchling. Showing the indifferent gonad. MGC-Mitotic germ cell, SC-Somatic cell. Scale bar is 50 µm.

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15 Day hatchlings

As a result of multiplication of germ cells,gonads increase in size with several germ cells. However, the gonads remain undifferentiated at this stage. (Fig 5)

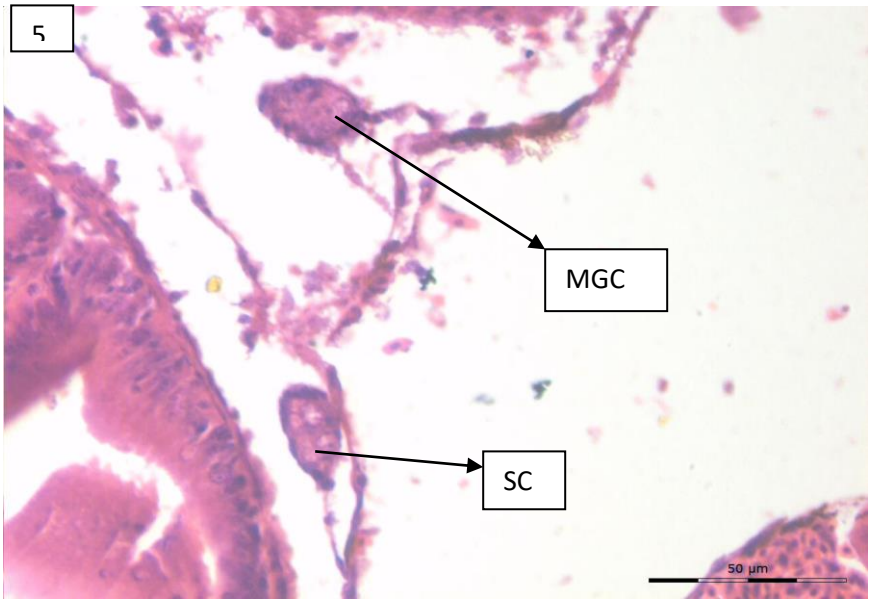

Figure 5 Section of 15day hatchling. MGC-Mitotic germ cell, SC-Somatic cell. Scale bar is 50 µm

20 Day hatchlings

Observation made in 20 day hatchlings reveals the presence of few clusters of gonial cells and there is a change in somatic tissue. Some gonial cells are large in size and show initiation of meiosis. . (Fig 6)

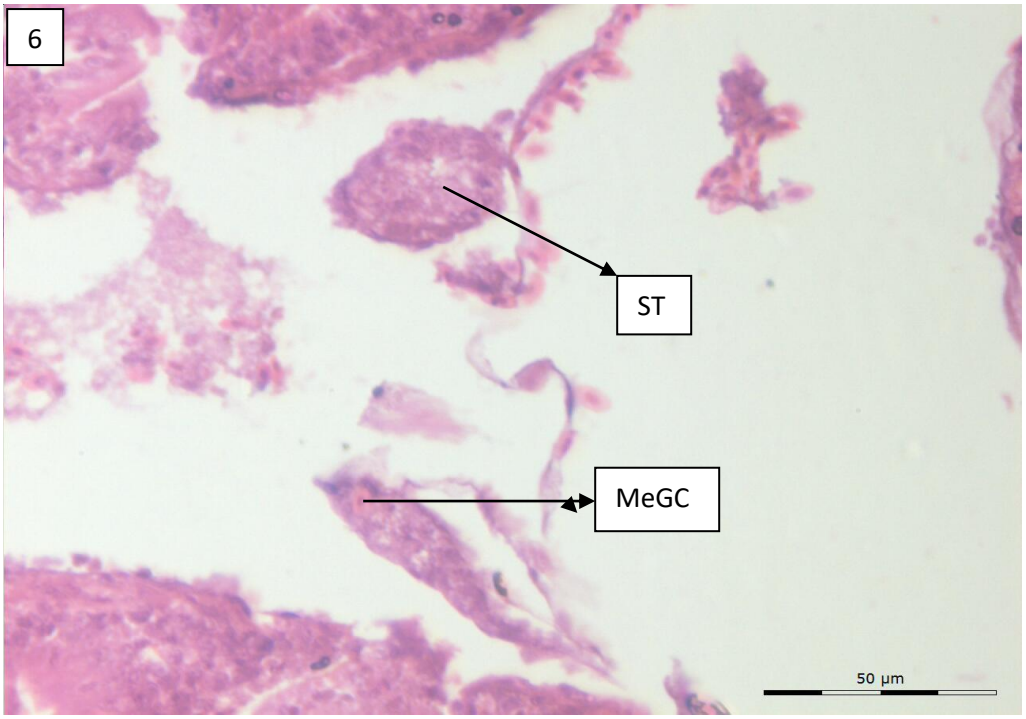

Figure 6 Section of 20day hatchling. MeGC-Meioticgerm cell, ST-Somatic tissue. Scale bar is 50 µm.

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25 Day hatchlings

Gonads show more number of gonial cells with meiotic stages, but there is no appearance of any cavity in the gonad. (Fig 7)

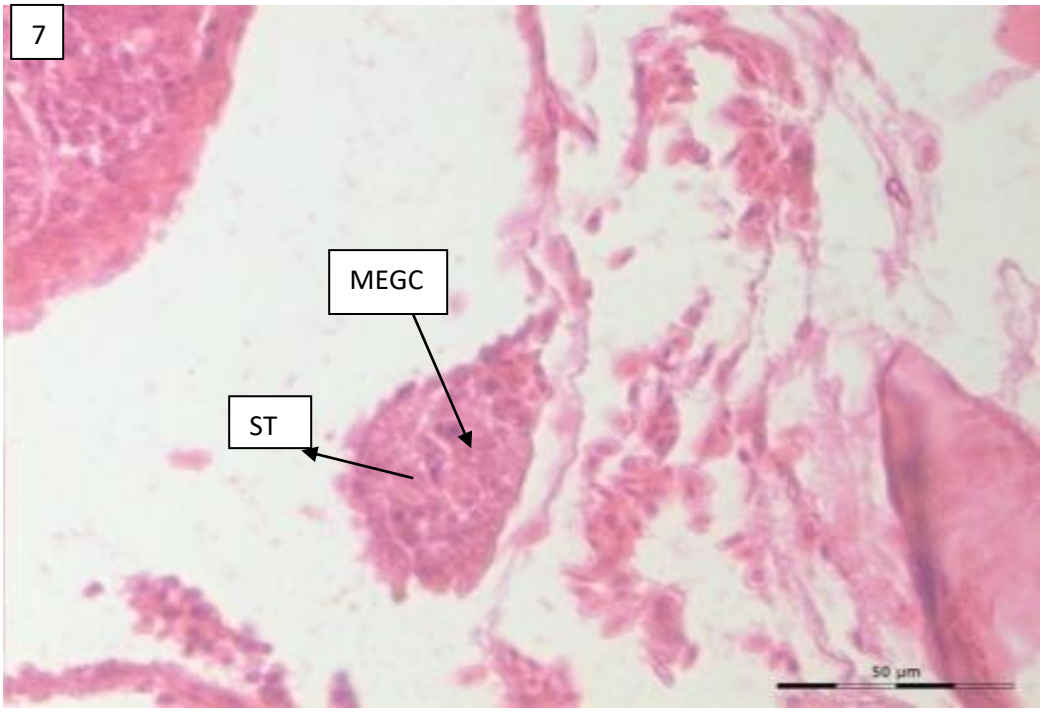

Figure 7 Section of 25day hatchling. MeGC-Meioticgerm cell, ST-Somatic tissue. Scale bar is 50 µm.

30 Day hatchling

There is an increase in the size of gonad due to mitotic activity, gonial cells with meiotic stages are more in number. (Fig 8)

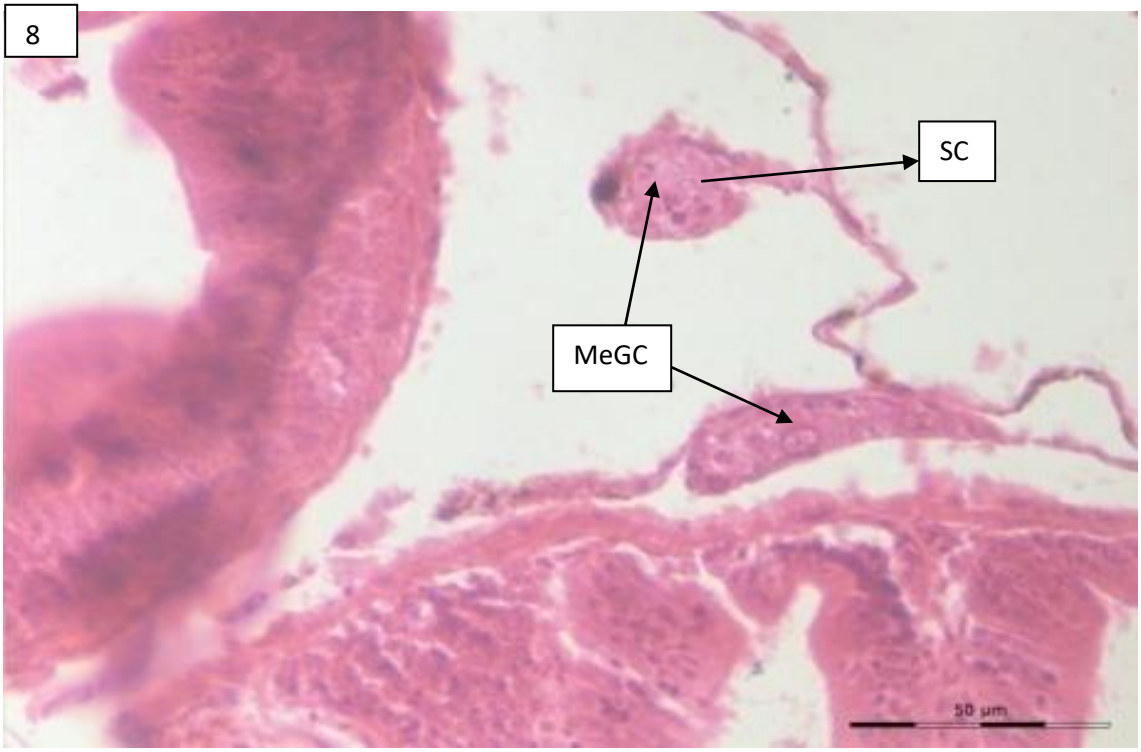

Figure 8 Section of 30day hatchling. MeGC-Meioticgerm cell. A scale bar is 50 µm.

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4. DISCUSSION

From the present histological observation on the gonadal development in the cichlid fish, Oreochromis mossambicus it was clear that morphological sex differentiation of gonad in this species occurred during the period between 20 to 25 days of age. Gonads in Sarotherodon tilapia, in the same species differentiate at15-30 days of post fertilization (Jalbert and zohar 1982).

In the same species Nakamura(1973)mentioned that gonadal sex differentiation took place between 16 and 20 days of age and Clemens and Inslee(1968) mentioned that gonadal sex differentiation took place between 35 and 48 days after hatching. Present finding is little close with the findings of Nakamura(1973) and very much similar to findings of Jalbert and Zohar.(1982) However the difference observed is not explainable on account of the lack of detailed description, and one of the causes is surmised to lie in morphological criteria adopted to decide the occurrence of sex differentiation of gonad.

In the present study no sexual differences in the morphology of somatic cells in the gonad were observed The gonads were in indifferent stage at 19 days of age, but in 20days of hatching some cysts of germ cells appear to show the meiotic prophase stage in some gonads, but in some the germ cells showed no change in their nuclear component. It was evident from these studies that the gonads with meiotic germ cells, ensured the formation of ovary or ovarian differentiation whereas the others would develop in to a male gonad. There was no formation of ovarian cavity in oreochromis mossambicus.Similar studies were reported in ovaries of some teleosts such as Lebistes reticulates (Goodrich et al, 1934) and Carassius auratus (Stromsten, 1931). However, formation of ovarian cavity is initiated with the start of oogenesis in fishes like Cottus bairdii (Hann 1927) and in Tilapia aurea (Eckstein and Spira 1965), when the germ cells are still in a sexually indifferent phase. However, the formation of ovarian cavity mentioned in the work of Nakamura (1973) and Clemens and Inslee, (1968) was not observed in the present work.

In various gonochoristic teleosts observed so for morphological differentiation of ovary emerge from sexually indifferent state, first evidently, preceding that of testis. In the present study the gonadal sexes were clearly distinguishable by 20 days of age when some cysts of germ cells appeared to enter the meiosis in some gonads. Thus germ cell maturation in female gonads occurs in evident advance than in male gonad in this species as in other teleostian fishes. Gonadal development in carp, Cyprinus carpio is of differentiated type, gonads are distinguished in to three types with respect to anatomical characteristics and morphology of germ cells from 10 weeks onwards. In C.carpio female and male gonads are distinguished at the age of 16 weeks (Nayak and Nadkarni, 1993), similar type of gonadal development has been observed in the gold fish, Carassius auratus by stromsten, (1931) and in the rosy barb, Barbus conchonius by Timmermans and Tavarne (1983). The gonad is in indifferent stage in 50 day old fish Catla catla and Labeo rohita, (Nayak and Nadkarni (1993). In Tilapia zilli male and female gonad can be distinguished at an early stage of 15 days. Differenciation of primordial gonad in to testis takes place at a later stage than that of the ovary (Yoshikawa and oguri, 1978) in the fish Tilapia zilli. In Tilapia mossambica(Sarotherodon mossambica) in which the ovarian differentiation occurs 20 days after hatching, its initiation is determined by both the meiotic process of germ cells and the formation of ovarian cavity (Nayak and Nadkarni 1993) Mode of formation of ovarian cavity varies greatly among the teleosts.

The PGCs aggregate to lie against the gonadal epithelium prior to the formation of gonad in Oreochromis mossambicus. Similar studies are reported in Channa punctatus (Belsare, 1966). The sex is differentiated suddenly in Channa punctatus (Belsare 1966) and other fishes .Similar studies are reported for Micropterus salmoides (Johnston, 1951).

Generally, for differentiated gonochoristic teleost fish, the occurrence of meiotic germ cells for oogenesis subsequent to the increase of germ cells is now accepted as the criterion of initial ovarian differentiation, such as in the medaka Oryzias latipes (Yamamoto, 1958), stickleback Gasterosteus aculeatus (Shimizu and Takahashi, 1980), rainbow trout Oncorhynchus mykiss (Lebrun et al., 1982), pejerrey Odontesthes bonariensis (Strüssmann et al., 1996) and annual fish Austrolebias charrua (Arezo et al., 2007). While for undifferentiated gonochorists, which exhibit juvenile hermaphroditism, the appearance of pre-meiotic oocytes is not always a reliable criterion by which to judge differentiation in these species, such as in zebra fish Danio rerio (Takahashi, 1977; Maack and Segner, 2003). Therefore, in these fishes, histological evidence indicating the formation of the ovarian cavity may be a reliable criterion for identifying the gonad as an ovary.

In grass carp Ctenopharyngodon idella and rainbow trout sex differentiation starts 50days or more after hatching (Jenson et al 1983, Van den hurk et al.1982) and in mugil auratus it starts at the end of one year, in some species golden grey mullet mugil auratus the indifferent stage can last for several years (chan and yeung, 1983).

Thus it is concluded that in Oreochromis mossambicus the gonads which are sexually in different at least in histological sense may initiate their sex differentiation at some time between 20 to 30 days of age.

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Declaration of conflicting interests

The authors declare that there are no conflicts of interests.

Data and materials availability

All data associated with this study are present in the paper.

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